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Bradykinin causes endothelium-independent hyperpolarisation and neuromodulation by prostanoid synthesis in hamster mesenteric artery

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Abstract

The mechanism of bradykinin-induced hyperpolarisation and purinergic neuromodulation was examined in the hamster superior mesenteric artery using intracellular microelectrode techniques. Bradykinin induced a concentration-dependent hyperpolarisation both in endothelium-intact and -denuded preparations. Indomethacin blocked this hyperpolarisation. Prostacyclin and iloprost also hyperpolarised the membrane of mesenteric artery, while prostaglandin E_2 did not evoke any membrane hyperpolarisation. The bradykinin-, prostacyclin- and iloprost-induced hyperpolarisation were inhibited by glibenclamide. Bradykinin also inhibited the amplitude of the purinergic excitatory junction potentials (e.j.p.s), both in endothelium-intact and -denuded preparations. Indomethacin blocked this inhibitory effect. Prostaglandin E_2 inhibited the e.j.p. in a concentration-dependent manner. Focally applied ATP-induced depolarisation was not modified by bradykinin or prostaglandin E_2. These findings suggest that bradykinin via prostanoids production pre-synaptically, inhibit the amplitude of purinergic e.j.p., resulting inhibitory purinergic neuromodulation. In addition, bradykinin-released prostanoids elicits membrane hyperpolarisation of smooth muscle cells through opening of K_{ATP} channels.

Keywords: Purinergic neuromodulation; Endothelium; Bradykinin; Prostanoid

1. Introduction

Bradykinin is a potent endogenous vasoactive polypeptide, known to act on both the endothelium (Nagao and Vanhoutte, 1992) and smooth muscle of blood vessels (Persson and Andersson, 1998) as well as on nerve endings of the vas deferens (Llona et al., 1987). Bradykinin releases prostaglandins, nitric oxide (NO) and other substances, such as the yet unidentified endothelium-derived hyperpolarising factors (EDHF) (Félétou et al., 1994), from the vascular endothelium to cause relaxation in different vessels (Ignarro et al., 1987; Ohlmann et al., 1997; Zhu et al., 1997). However, an intact endothelium is not required for the relaxation of the rabbit coeliac artery to bradykinin (Cherry et al., 1982; Förstermann et al., 1986). Similarly, endothelium-independent contractions to bradykinin have been reported in the guinea-pig anterior mesenteric vein (Gaudreau et al., 1981), rabbit jugular vein (Calixto and Medeiros, 1992), rabbit mesenteric vein (Regoli et al., 1977) and porcine ileal artery (Persson and Andersson, 1998). Bradykinin is also known to be active in the peripheral nervous system. It excites sympathetic ganglia (Feldberg and Lewis, 1964; Lewis and Reit, 1965, 1966) and releases catecholamines from the adrenal medulla (Staszewska-Barczak and Vane, 1967). In contrast, Starke et al. (1977) reported that bradykinin inhibits the release of the noradrenaline from the sympathetic nerves. More recent evidence shows that bradykinin increases the release of noradrenaline in isolated atria (Chulak et al., 1995, 1998). However, to our knowledge, there is no information available concerning the mechanisms involved in the neuromodulatory effect of bradykinin on the purinergic component of sympathetic neurotransmission. As an experimental model, we used the hamster mesenteric artery, a tissue where e.j.p.s are induced by the activation of P2X purinoceptors (Thapaliya et al., 1999a). In separate experiments conventional intracellular recording techniques have been used to monitor electrically evoked excitatory junction potentials (e.j.p.s), which provide a measure of ATP release (McLaren et al., 1995; Brock and Cunnane, 1999).

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Thus, the aims of the present study were: (1) to explain the mechanism of bradykinin-induced membrane hyperpolarisation; (2) to explore if bradykinin could modulate purinergic neurotransmission as judged from the amplitude of electrically evoked e.j.p.s; and (3) to investigate whether bradykinin exerts its action at the post-junctional level, by determining its effects on exogenously applied ATP-induced depolarisations. Some of the results were reported to the Japanese Pharmacological Society (Thapaliya et al., 1999b).

2. Materials and methods

2.1. Tissue preparation

Male Golden Syrian hamsters weighing 100–130 g were killed by overdose of diethyl ether after administration of heparin (100 U) into the left ventricle of heart and exsanguinated, following a protocol approved by the Gifu University, Animal Care and Use Committee in accordance with Japanese Department of Agriculture guidelines. The superior mesenteric artery was carefully dissected from ileal region and placed in Physiological Salt Solution (PSS) at room temperature. The adherent tissues were removed and vessels were cannulated at the proximal end with glass micropipette (200 μm tip diameter) attached to the gravity-driven perfusion apparatus and perfused the vessel with warmed (35°C) PSS to remove the blood in the vessels. Care was taken to ensure that the endothelium was not damaged during processing of the preparation. When required, endothelial cells were removed by injecting warmed (35°C) PSS containing the collagenase (1 mg ml^{-1}) in the perfusion route for 15 min. The successful removal of endothelial cells was confirmed by the absence of the typical hyperpolarisation response to acetylcholine. To observe changes in the membrane potential, arteries of about 100–350 μm (outside diameter) were used.

2.2. Electrophysiological experiments

The arteries were placed in the partition chamber in which large extracellular silver–silver chloride plates were used to elicit nerve stimulation, as described previously (Bolton et al., 1984). The preparation was superfused at a constant flow rate (3 ml min^{-1}) with warmed (35°C) PSS. Membrane potentials were recorded using a conventional microelectrode technique, using glass capillary microelectrodes filled with 3 M KCl with tip resistances ranging from 50 to 100 MΩ. Excitatory junction potentials were evoked by electrical stimulation of perivascular nerves of the tissue with a square-wave pulse of 1 ms in duration at supramaximal intensity delivered by a stimulator (SEN-3301, Nihon Kohden, Tokyo, Japan). Nerve stimulation was applied using 0.25 Hz frequencies in a train. Impalements were made from the adventitial side, within 2 mm from the stimulation electrode. The electrical activities were monitored on an oscilloscope (CS 4026, Kenwood, Tokyo, Japan) and recorded on a thermal-array recorder (RTA-1100 M, Nihon Kohden) and on a PCM data recorder (RD-111T, TEAC, Tokyo, Japan) to allow replay for further analysis.

2.3. Pressure ejection of ATP

To apply small quantities of ATP to localized regions and at desired particular time, the drugs were pressure-ejected from a micropipette. A Pneumatic PicoPump was used for this purpose with a pressure of 10 psi and pulse duration of 5 ms. Fiber-filled glass micropipette (outside diameter = 1 mm, inside diameter = 0.5 mm) were drawn with a microelectrode puller (Narishige, Japan, Type PP-83) into the fine tips. The outside diameter of tip was 12.8 ± 1.0 μm (15 pipette measured). Pipette was then filled with a 10^{-2} m solution of ATP. To avoid possible disensitisation due to leakage of drugs, the pipette were maintained at a distance from the preparation and positioned next to the electrode only after a stable impalements was obtained; drug application was initiated thereafter. After application of drug, the pipette was withdrawn.

2.4. Drugs and solutions

The composition of the physiological salt solution was (mM): Na^+ 137; K^+ 5.9; Ca^{2+} 2.5; Mg^{2+} 1.2; Cl^- 134; HCO_3^- 15.4; $H_2PO_4^-$ 1.2; glucose 11.4. The solution in the supply reservoir was gassed continuously with a 95% O_2/5% CO_2 gas mixture creating a pH of 7.2 and was warmed to 33–35°C. The drugs used were as follows: Bradykinin acetate salt, prostaglandin E_2, prostacyclin, collagenase, tetrodotoxin, acetylcholine chloride, N^{ω}-nitro-L-arginine methyl ester, apamin, charybdotoxin (Sigma); Iloprost (Schering Aktiengesellschaft, Germany); Glibenclamide and indomethacin (RBI); tetraethylammonium chloride (Wako, Japan). Indomethacin (10 mM) was dissolved in an equimolar concentration of Na_2CO_3. All other drugs were dissolved in distilled water. The drugs were serially diluted in the PPS solution to the required final concentrations just before the experiments.

2.5. Statistics

Data are shown as mean ± S.E.M.; *n* indicates the number of separate arteries in which electrical events were recorded. Statistical analysis was performed with Student's unpaired *t*-test, and a *P* value of < 0.05 was regarded as significant.

3. Results

The smooth muscle cells of hamster superior mesenteric arteries had resting membrane potentials of -63.9 ± 0.7 mV ($n = 125$), and cells were electrically quiescent when unstimulated.

3.1. Membrane hyperpolarisation produced by bradykinin

Bradykinin (10^{-10} to 10^{-5} M) induced a concentration-dependent membrane hyperpolarisation (Fig. 1). The threshold concentration of bradykinin for the hyperpolarisation was 5×10^{-10} M. The involvement of endothelium-derived relaxing factor/nitric oxide in the bradykinin-induced hyperpolarisation was also investigated. Treatment with nitric oxide synthase inhibitor, L-NAME (10^{-4} M) for 30 min did not significantly change the resting membrane potential (-64.1 ± 0.9 mV, $n = 5$, $P > 0.05$). In the presence of this enzyme inhibitor, the bradykinin-induced membrane hyperpolarisation also was not significantly modified (Fig. 2A).

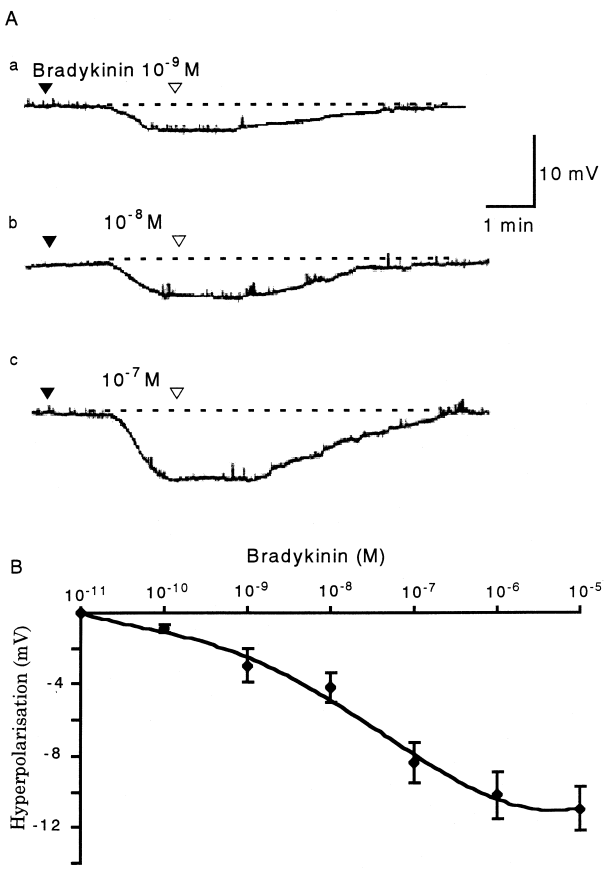

Fig. 1. (A) Typical recordings of membrane potential showing that bradykinin (10^{-9} to 10^{-7} M) evoked a concentration-dependent hyperpolarisation. Onset (▼) and washout (▽) of drug application. The membrane potentials of (Aa), (Ab) and (Ac) were −63, −62 and −65 mV, respectively. (B) Summary of concentration-dependent hyperpolarisation evoked by bradykinin (10^{-11} to 10^{-5} M). Values are expressed as means ± S.E.M. ($n = 9$–11).

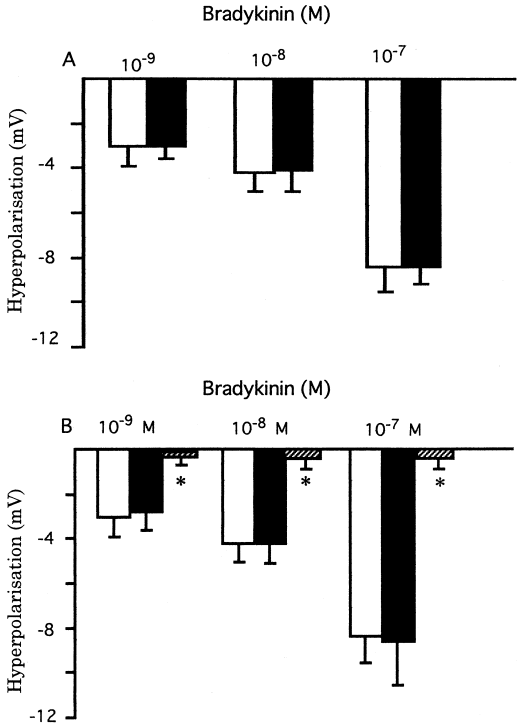

Fig. 2. (A) Membrane hyperpolarisation to bradykinin (10^{-9} to 10^{-7} M) (open bars) and the effect of L-NAME (10^{-4} M) (filled bars), and (B) endothelium denudation (filled bars) and indomethacin (5×10^{-6} M) (hatched bars). Values are expressed as means ± S.E.M. ($n = 7$–12). Treatment with indomethacin strongly inhibited bradykinin-induced hyperpolarisation, but L-NAME and endothelium denudation did not. Significantly different from control $^{*}P < 0.001$.

The involvement of endothelium in the bradykinin-induced hyperpolarisation was studied. Endothelium-denuded smooth muscle cells of the distal branches of superior mesenteric arteries were significantly depolarised from

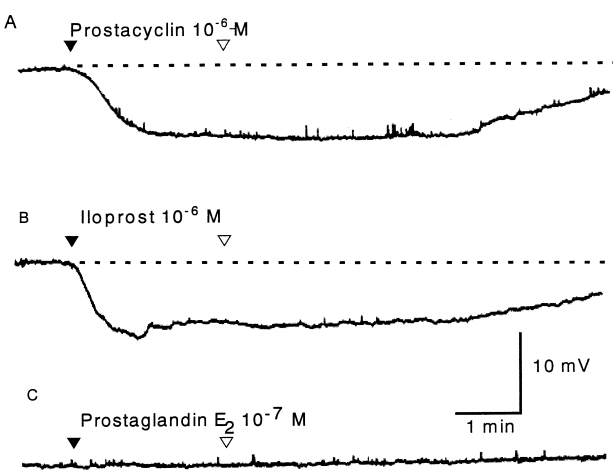

Fig. 3. Typical recordings of membrane potential showing that (A) prostacyclin (10^{-6} M) and (B) iloprost 10^{-6} M) hyperpolarised the membrane, but (C) PGE_2 (10^{-7} M) did not. Onset (▼) and washout (▽) of drug application. Membrane potentials for (A), (B) and (C) were −64, −63, and −61 mV, respectively.

Table 1
Effect of potassium channel inhibitors on bradykinin-, prostacyclin- and iloprost-induced hyperpolarisation

	Membrane potential (mV)	Hyperpolarisation (mV)		
		Bradykinin (10^{-7} M)	Prostacyclin (10^{-6} M)	Iloprost (10^{-7} M)
Control	-62.6 ± 0.6 ($n = 35$)	-8.3 ± 1.4 ($n = 12$)	-9.5 ± 1.0 ($n = 8$)	-8.3 ± 1.8 ($n = 9$)
+ Tetraethylammonium (10^{-3} M)	-61.3 ± 1.6 ($n = 7$)	-8.5 ± 1.7 ($n = 5$)	-8.9 ± 1.3 ($n = 4$)	-7.0 ± 1.0 ($n = 7$)
+ Apamin (10^{-7} M)	-63.1 ± 1.1 ($n = 7$)	-7.9 ± 1.5 ($n = 5$)	-9.1 ± 0.9 ($n = 5$)	–
+ Charybdotoxin (10^{-7} M)	-63.9 ± 1.1 ($n = 5$)	-7.8 ± 1.1 ($n = 6$)	-8.7 ± 0.9 ($n = 5$)	–
+ Glibenclamide (10^{-6} M)	-54.5 ± 1.4 ($n = 8$)[a]	-1.5 ± 0.3 ($n = 5$)[b]	-0.9 ± 0.3 ($n = 4$)[b]	-0.9 ± 0.2 ($n = 7$)[b]

Hyperpolarisation measurements are expressed as means ± S.E.M.; n represents the number of arteries in which membrane potential was recorded.
[a] Significantly different from control values $P < 0.05$.
[b] Significantly different from control values $P < 0.01$.

-63.9 ± 0.7 ($n = 25$) to -60.3 ± 1.0 mV ($n = 10$, $P < 0.05$). However, the denudation of endothelium did not significantly change the bradykinin (10^{-9} to 10^{-7} M)-induced hyperpolarisation (Fig. 2B).

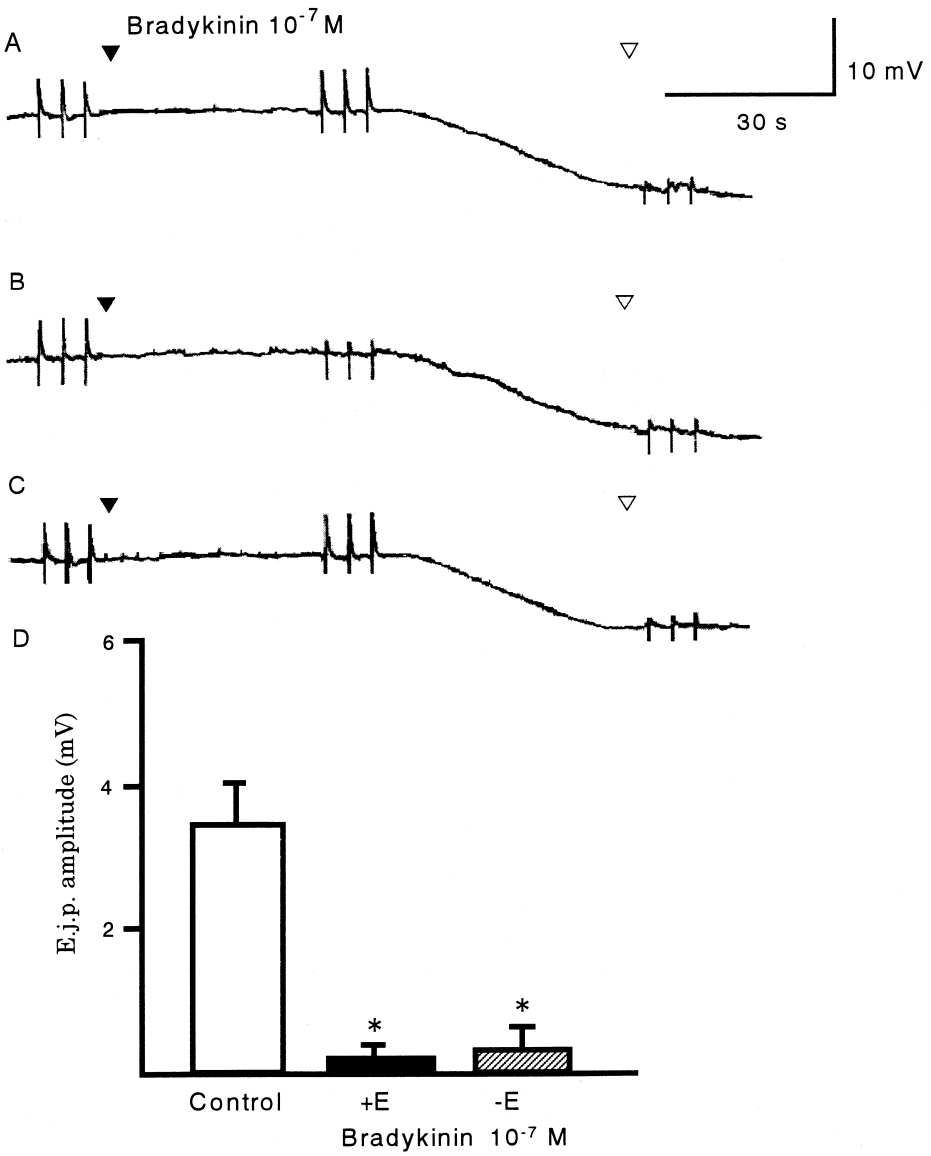

Fig. 4. Typical traces of effects of bradykinin (10^{-7} M) on amplitude of e.j.p. This peptide exhibited two types of responses on amplitude of e.j.p. (A) After 1 min of application, membrane was hyperpolarised and e.j.p. was also inhibited. (B) In the second type of preparation before the membrane hyperpolarisation, the e.j.p. was inhibited. (C) Bradykinin-induced e.j.p. inhibition was not modified in endothelium-denuded preparation. Onset (▼) and washout (▽) of drug application. (D) Summary of effect of bradykinin on e.j.p. amplitude in endothelium-intact (filled bars) and -denuded (hatched bars) preparations. Membrane potentials for (A), (B), (C) were -63 and -62, -64 mV, respectively. Significantly different from control $^{*}P < 0.001$.

3.2. Effect of indomethacin on bradykinin-induced hyperpolarisation

Whether or not involvement of prostanoids in the bradykinin-induced hyperpolarisation were tested. Treatment with the non-specific cyclooxygenase enzyme inhibitor, indomethacin (5×10^{-6} M) did not significantly change the resting membrane potential (-64 ± 0.3 mV, $n = 5$, $P > 0.05$). However, indomethacin strongly inhibited the bradykinin (10^{-9} to 10^{-7} M)-induced hyperpolarisation in endothelium-deduced (Fig. 2B), as well as endothelium-intact preparations ($n = 7$, data not shown). Na_2CO_3 (5×10^{-6} M) at the concentration used to prepare indomethacin (5×10^{-6} M) had no effect on either membrane potential or on the bradykinin-induced hyperpolarisation ($n = 4$, data not shown).

These results suggested that the hyperpolarisation produced by bradykinin was due to non-endothelial cyclooxygenase products (prostanoids). We also examined whether the main exogenous prostanoids (protacyclin, prostaglandin E_2) also produce responses similar to those evoked by bradykinin. Prostacyclin (10^{-7} and 10^{-6} M) produced a membrane hyperpolarisation of 2.6 ± 0.8 ($n = 4$) and 10.5 ± 1.0 mV ($n = 9$), respectively. Similarly, the synthetic prostacyclin analogue, iloprost (10^{-6} M), also produced membrane hyperpolarisation of 11.9 ± 1.5 mV ($n = 8$). However, prostaglandin E_2 (10^{-11} to 10^{-7} M) did not evoke any membrane hyperpolarisation (Fig. 3).

3.3. Type of K^+ channel involved in bradykinin and exogenous prostanoids-induced hyperpolarisation

Table 1 shows the effects of different potassium channel inhibitors on bradykinin, prostacyclin and iloprost-induced hypepolarisations. Treatment with tetraethyl-

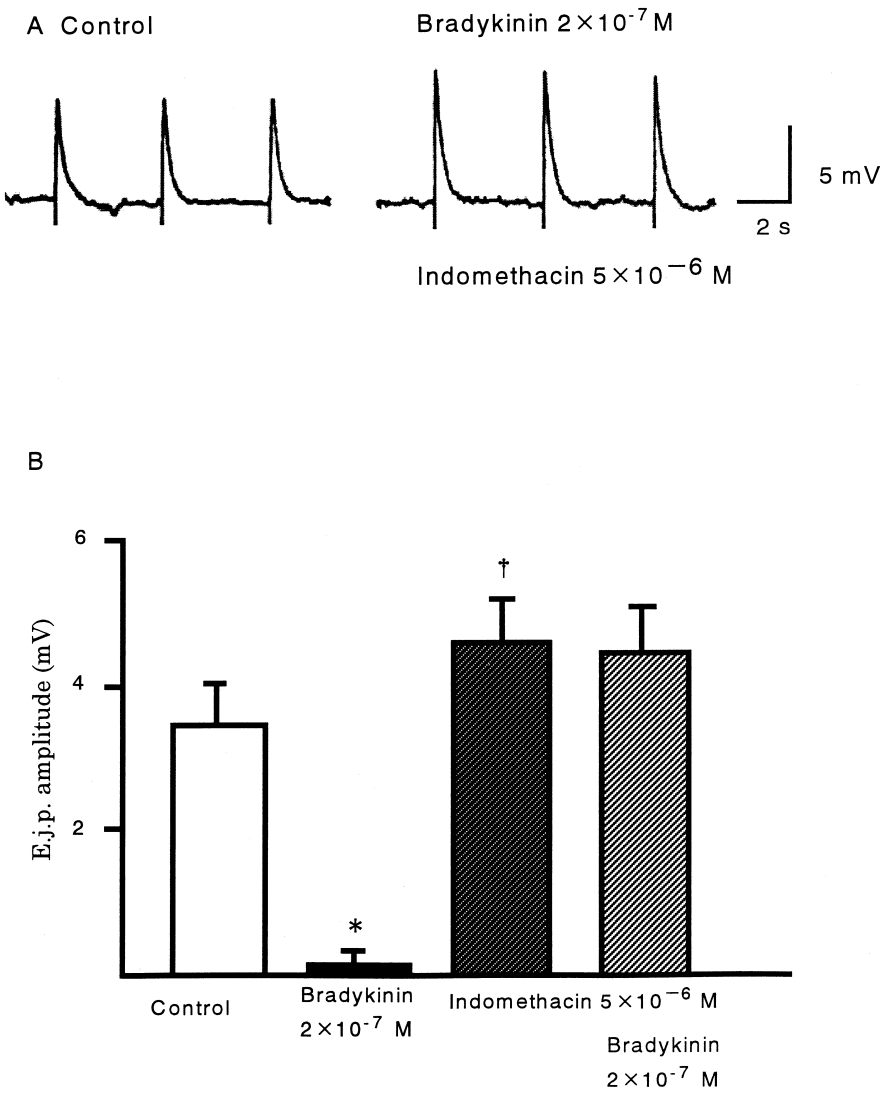

Fig. 5. (A) Typical traces of effects of indomethacin (5×10^{-6} M) on bradykinin (2×10^{-7} M)-induced e.j.p.s inhibition. Membrane potential was -65 mV. (B) Summary of effect of indomethacin on bradykinin-induced e.j.p.s inhibition. Values are expressed as means $\pm$ S.E.M. ($n = 7$–13). Significantly different from control $^{\dagger}P < 0.05$, $^{*}P < 0.001$.

ammonium (10^{-3} M), apamin (10^{-7} M) and charybdotoxin (10^{-7} M) did not change the membrane potential (see Table 1). In the presence of these potassium channel inhibitors, bradykinin-, prostacyclin-, and iloprost-induced hyperpolarisations were not modified. However, glibenclamide (10^{-6} M), which selectively inhibits the ATP-sensitive K_{ATP} channels, significantly depolarised the membrane. It also inhibited the membrane hyperpolarisation induced by bradykinin, iloprost and prostacyclin.

3.4. Effect of bradykinin on excitatory junction potentials evoked by trains of brief stimuli

Perivascular nerve stimulation at a rate of 0.25 Hz frequency evoked e.j.p.s. The amplitude of e.j.p.s ranged between 4 and 12 mV (6.0 ± 0.7 mV, $n = 105$). Experiments were conducted to investigate the effect of bradykinin (10^{-7} M) on e.j.p. amplitude. Fig. 4 shows the effects of bradykinin (10^{-7} M) on the e.j.p. amplitude. After about 1 min application of bradykinin, the e.j.p. was inhibited (Fig. 4A) in about 52% of preparations only during the development of membrane hyperpolarisations. In the remaining 48% of tissues there was inhibition of e.j.p. before the development of membrane hyperpolarisation to bradykinin (Fig. 4B). There was no significant difference in resting membrane potential between these cells, which showed the two different types of responses.

Whether or not endothelium was required in bradykinin-induced inhibition f e.j.p. amplitude was assessed. The denudation of endothelium did not significantly modify the bradykinin-induced inhibition of e.j.p. amplitude (Fig. 4C and D). These results implied that endothelium was not essential in bradykinin-induced e.j.p. inhibition.

3.5. Effects of endomethacin on bradykinin-induced e.j.p.s inhibition

The involvement of prostanoids in bradykinin-induced inhibition of e.j.p. amplitude was investigated with the use of indomethacin (5×10^{-6} M) for 30 min. Indomethacin significantly increased the amplitude of e.j.p.s. In these indomethacin-treated preparations bradykinin (2×10^{-7} M) did not inhibit the e.j.p. amplitude (Fig. 5).

Prostacyclin (10^{-7} M) did not significantly inhibit the e.j.p. amplitude. Ten times higher concentration of this agonist significantly inhibited the amplitude of e.j.p.s by 25% (Fig. 6Ab and Bb) and the time constant of decay of e.j.p. (τ_{decay}) was significantly reduced from 345.7 ± 17.0 to 285.8 ± 25.0 ms ($P < 0.01$). The inhibition of e.j.p. amplitude by iloprost (10^{-6} M) was similar to those observed by prostacyclin (30%, $n = 5$). The strong inhibition of the e.j.p. amplitude by bradykinin can therefore not be explained by prostacyclin only. Thus, the effects of prostaglandin E_2 on the e.j.p. amplitude were also studied. Prostaglandin E_2 (10^{-11} to 10^{-8} M) strongly inhibited the

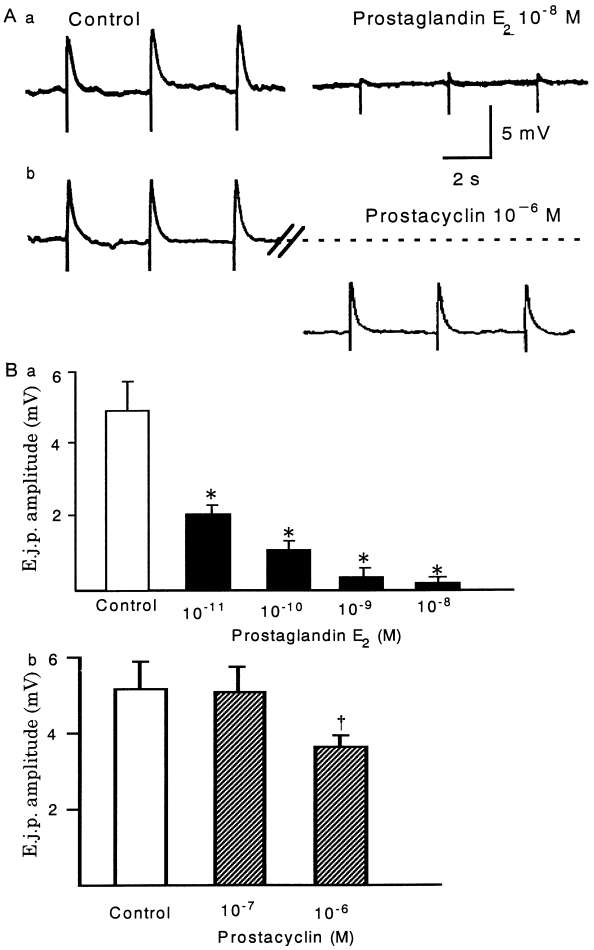

Fig. 6. (Aa and Ab) Typical recordings of effects of prostaglandin E_2 (10^{-8} M) and prostacyclin (10^{-6} M) on e.j.p. amplitude, respectively. (Ba and Bb) Summary of effect of prostaglandin E_2 and prostacyclin on e.j.p. amplitude, respectively. Values are expressed as means ± S.E.M. ($n = 7$–11). Significantly different from control $^{\dagger}P < 0.05$, $^{*}P < 0.001$.

e.j.p.s in a concentration-dependent manner (Fig. 6Aa and Ba). This inhibition was not modified by pre-treatment of preparations with indomethacin ($n = 6$, data not shown).

3.6. Effect of bradykinin and prostaglandin E_2 on ATP-induced depolarisation

Experiments were carried out to observe the effect of bradykinin and prostaglandin E_2 on the depolarisation responses produced by ATP, the objective being to determine the post-junctional action of bradykinin and prostaglandin E_2. When ATP (10^{-2} M) was pressure ejected (10 psi, 10 ms duration pulse) in small quantities from a micropipette, positioned about 200 μm from the recording electrode, a membrane depolarisation of 14.5 ± 1.0 mV ($n = 10$) was recorded. When ATP was applied during the bradykinin (10^{-9} to 10^{-7} M)-induced hyperpolarisation, the depolarising response of the smooth muscle cells to ATP was not inhibited (14.9 ± 1.3 mV, $n = 4$, $P > 0.05$). Prostaglandin E_2, at concentrations between 10^{-9} and

10^{-7} M, also did not modify the depolarisation evoked by ATP (14.9 ± 1.1 mV, $n = 5$, $P > 0.05$).

4. Discussion

Our experiments have demonstrated that the bradykinin-induced hyperpolarisation and modulation of purinergic neurotransmission in the isolated hamster mesenteric arteries is an indirect effect, mediated by the release of prostaglandins. Many studies suggest that bradykinin-induced hyperpolarisation or relaxation is endothelium-dependent, even though there is a considerable regional and species heterogeneity. In the superior mesenteric artery of man and dog, bradykinin-induced relaxation is entirely dependent on the presence of endothelial cells (Cherry et al., 1982). Félétou et al. (1994) have reported that bradykinin is a powerful endogenous endothelium-dependent vasodilator, releasing prostaglandins, nitric oxide and other substances such as the so far unidentified EDHF. Furthermore, in the pig coronary artery bradykinin-induced hyperpolarisation is solely mediated by EDHF (Nagao and Vanhoutte, 1992). In contrast, only NO is involved in the arteriolar dilatation component of the bradykinin-induced increase of microvascular permeability in hamster cheek pouch (Félétou et al., 1996). On the other hand, other authors have reported that endothelial cells are not necessary for bradykinin-induced relaxation of cat mesenteric (Cherry et al., 1982) and rabbit coeliac artery (Cherry et al., 1982; Förstermann et al., 1986; Ritter et al., 1989). In the present study, the hyperpolarisation induced by bradykinin is also endothelium-independent. Both prostaglandins and bradykinin hyperpolarised the membrane potential of hamster mesenteric arteries. Furthermore, the bradykinin-induced hyperpolarisation was completely blocked by indomethacin. These results strongly suggest that cyclooxygenase products probably prostaglandins mediate the hyperpolarisation caused by bradykinin. Previously, endothelium-independent responses to bradykinin have been considered to be mediated by prostaglandin release, based on the result that these responses were inhibited by the cyclooxygenase inhibitor, indomethacin (Cherry et al., 1982; Förstermann et al., 1986; Ritter et al., 1989).

Vascular smooth muscle cells express different types of potassium channels (Kuriyama et al., 1995). ATP-sensitive potassium (K_{ATP}) channels are blocked by glibenclamide and large conductance Ca^{2+}-activated (BK_{Ca}) channels by low concentration of tetraethylammonium or by charybdotoxin. In the mesenteric artery of hamster, prostacyclin- and iloprost-induced hyperpolarisation was sensitive to glibenclamide, indicating the activation of K_{ATP} channels (Jackson et al., 1993; Parkington et al., 1993; Miyoshi et al., 1994; Plane et al., 1995; Corriu et al., 1996). In this study, bradykinin-induced hyperpolarisations were also blocked by glibenclamide, further supporting the notion that the bradykinin-induced response is mediated by prostaglandin synthesis.

ATP released in response to sympathetic nerve stimulation, is thought to act on P2X purinoceptors on smooth muscle cells to evoke transient depolarisation, known as excitatory junction potentials in vascular (Brock and Cunnane, 1999) and non-vascular tissues (Brock and Cunnane, 1996). In such preparations intracellularly recorded e.j.p.s were used as a measure of ATP release (Sneddon and Burnstock, 1985; Mclaren et al., 1995; Brock and Cunnane, 1996, 1999). In our previous report, e.j.p.s recorded from hamster mesenteric arteries are inhibited by P2X purinoceptor antagonist, pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS) or α β-MeATP, thus, these e.j.p.s are believed to be mediated by ATP (Thapaliya et al., 1999a). In the present experiments, we indirectly measured the ATP release, as judged from the amplitude of e.j.p.s.

In the present experiment, bradykinin inhibited the purinergic e.j.p.s, and this inhibition was endothelium-independent. In the presence of cyclooxygenase inhibitor, indomethacin, bradykinin din not inhibit e.j.p. These findings suggest that prostaglandins inhibit the purinergic neurotransmission in hamster mesenteric artery. Previously, Brock and Cunnane (1996) have also reported that prostaglandin E_2 powerfully inhibited quantal ATP release in the guinea-pig vas deferens. The type of prostaglandin involved in this inhibition also might be Prostaglandin E_2. Among the exogenous prostaglandins the minimum concentration of prostaglandin E_2 required to suppress e.j.p. amplitude was 10^{-11} M. In contrast, 10^{-6} M prostacyclin only slightly inhibited the e.j.p., but was more potent than prostaglandin E_2 in evoking the membrane hyperpolarisations. From these results we conclude that more than one type of prostaglandin are synthesised by bradykinin in hamster mesenteric artery and prostaglandin E_2 was mainly involved the in inhibition of purinergic neurotransmission. Previously, it is reported that in rabbit mesenteric arteries, both prostacyclin and prostaglandin E_2 synthesis are increased in response to bradykinin in an endothelium-independent manner (Förstermann et al., 1986).

It is reported that bradykinin acts both pre- and postsynaptically (Llona et al., 1987) in sympathetic neuromodulation. However, in the present experiments, ATP-induced depolarisation in smooth muscle cells was not modified by bradykinin or prostaglandins, supporting that the bradykinin-induced e.j.p. inhibition does not occur at the post-junctional level. Bradykinin induces a hyperpolarisation, and previous observations have suggested that hyperpolarisation is likely to result from an increase in potassium conductance in vascular smooth muscle cells (Bolton et al., 1984; Corriu et al., 1996). Such increased potassium conductance may prevent depolarising responses of the smooth muscle cells, resulting in reduction of e.j.p.s amplitude. Thus, e.j.p. amplitude might decrease either when amount of transmitter released from the perivascular nerve

or τ_{decay} decreases (Kotecha and Neild, 1995). In the present experiments, occasionally, bradykinin inhibited the e.j.p.s before the initiation of membrane hyperpolarisation, and prostaglandin E_2 always inhibited the e.j.p. without any change in membrane potential. Thus, in bradykinin-induced e.j.p. inhibition, change in muscle properties might not be involved. The mechanism of inhibition of e.j.p. by bradykinin-mediated prostaglandins may be due to inhibition of Ca^{2+} influx to the nerve terminals as it is reported that prostaglandins mainly suppress chemical transmitter release from nerve terminals due to interaction with Ca^{2+} influx (Kuriyama and Makita, 1982), further supporting that the action of bradykinin via prostaglandin synthesis is prejunctional. Taken together, our findings suggest that bradykinin via the cyclooxygenase products, acting prejunctioally, inhibit the ATP release in hamster mesenteric artery.

Nerve stimulation of $\leqq 1$ Hz, in hamster mesenteric artery evoked noradrenergic slow depolarisation (Thapaliya et al., 1999a). Hashitani et al. (1998) have also reported that low-frequency (0.5 Hz) stimulation evoked purinergic e.j.p. and high-frequency (10 Hz) stimulation evoked noradrenergic slow depolarisations. In the present experiments, we used only low-frequency (0.25 Hz) stimulation, which did not evoke slow depolarisations. Thus, we could not assess the neuromodulatory effect of bradykinin on noradrenaline release. However, others have reported that bradykinin increases the release of noradrenaline in rat (Chulak et al., 1995) and human atria (Rump et al., 1997). In contrast, Starke et al. (1977) reported that bradykinin inhibits noradrenaline release elicited by sympathetic nerve stimulation, perhaps because in that tissue, stimulation of prostaglandin production by bradykinin and subsequent inhibition of noradrenaline release by prostaglandin prevails. Similarly, facilitatory effect of bradykinin on noradrenaline release is further enhanced when the preparations were treated with cyclooxygenase inhibitors (Chulak et al., 1998).

In conclusion, this study provides evidence that bradykinin hyperpolarises the hamster mesenteric artery by stimulating release of prostaglandins from sources other than endothelial cells. These prostaglandins cause inhibitory purinergic neuromodulation.

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